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USAF School of Aerospace Medicine, Brooks AF Base, Tex.

SAM-TDR-63-73. CONTROLLED CONTAMINATION: A PRACTICAL APPROACH FOR DEVELOPING STERILIZATION PROCEDURES FOR SEALED COMPONENTS OF SPACECRAFT. Sept. 63, 6 pp. incl illus, tables, 8 refs.

Unclassified Report

Deliberate contamination of components during manufacture appears both practical and feasible for developing sterilization procedures for spacecraft components. Thus, it is possible to determine whether normal manufacturing procedures are

1. Sterilization of spacecraft components
2. Microbiology

I. AFSC Task 775302
II. J. T. Cordaro; H. Buchanan; B. Mann; A. K. Miller
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FOREWORD

This report was prepared by the following personnel:

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The *Bacillus stearothermophilus* FS1518 spores were obtained from the National Canners Association through the courtesy of William E. Perkins.

The *Bacillus subtilis* var. *niger* was obtained from Food, Science, and Technology Division, M. I. T., through the courtesy of N. S. Davis.

ABSTRACT

Deliberate contamination of components during manufacture appears both practical and feasible for developing sterilization procedures for spacecraft components. Thus, it is possible to determine whether normal manufacturing procedures are sufficient to sterilize or whether the sterilization procedures required (e.g., temperature-time intervals for dry heat) to sterilize can be accomplished without component damage. Methods are presented for controlled contamination with bacterial spores highly resistant to dry heat and bacteriologic recovery of such spores. Impregnated (e.g., with polybutylene) capacitors were rendered sterile during manufacture; nonimpregnated capacitors were not. Any damaging effects of heat sterilization might be increased if the components were subjected to further heating when installed in circuits of spacecraft instrumentations.

This technical documentary report has been reviewed and is approved.

Robert B Payne

ROBERT B. PAYNE
Colonel, USAF, MSC
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CONTROLLED CONTAMINATION: A PRACTICAL APPROACH FOR DEVELOPING STERILIZATION PROCEDURES FOR SEALED COMPONENTS OF SPACECRAFT

1. INTRODUCTION

A special problem in spacecraft sterilization concerns the interior of sealed electronic components. Obviously gaseous disinfectants cannot be used, and impairment of function may occur with methods involving either heat or radiation or both (3). A factor of prime importance in this problem, as in any sterilization problem, is the level of bacterial contamination. Preliminary studies on the existence of contamination in sealed components have shown that natural contamination is sporadic and of low density (1, 7, 8); hence, natural contamination cannot be used as a basis for developing sterilization procedures.

Many components are exposed during manufacture to temperature-time intervals that would appear, a priori, sufficient for sterilization. Temperature-time data for killing bacteria are usually obtained, however, by exposing spores dried on the surfaces of such materials as glass, polished metal, filter paper, etc. Recently, bacterial spores in sand, vermiculite, soil, etc., were reported by Koesterer and Bruch (5) and Koesterer (6) to have resistances somewhat higher than expected. The same spores in the interior of electronic components might have still different resistances.

In view of these considerations, it was recommended from this laboratory (2) that sterilization procedures for sealed components be developed by the use of components deliberately contaminated during manufacture. With this approach it was believed that it would be possible to determine sterilizing potential of normal manufacturing processes or the additional regimen required (e.g., dry heat, temperature-time intervals, radiation dosage, or all of these) to sterilize the components without affecting operational characteristics.

This report presents the results of an investigation performed as a joint in-house and contractual research program.[1] The experimental approach included the following:

1. Selection of appropriate microorganisms with high resistance to dry heat, chemicals, or radiation, or to all of these.

2. Contamination during manufacture of the interior of components with standard high-cell concentrations (10^5 to 10^6).

3. Performance tests of assembled contaminated components.

4. Exposure of contaminated components to dry heat or radiation regimens or both.

5. Performance tests on treated contaminated components.

6. Bacteriologic examination of undamaged components.

The contractual research effort involved vendor contact, selection of representative types of components presently installed in current spacecraft (e.g., Ranger and Surveyor), and controlled contamination of components. Exposure to dry heat, performance characteristic tests, and bacteriologic analyses were performed at the School of Aerospace Medicine.

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[1]Contractor: Lockheed Missiles & Space Company, Spacecraft Sterilization Systems, Van Nuys, Calif. Contract No. AF 41(609)-1544.

2. MATERIAL AND METHODS

Selection of microorganisms

Controlled contamination of components during manufacture necessitates the use of microorganisms highly resistant to either dry heat or radiation or both to assure acceptable confidence levels of sterility (99.99%). *Bacillus stearothermophilus* FS1518 spores were used at the beginning of this study, since they are frequently employed as an index of sterilization efficiency. Furthermore, these spores require incubation at 55° or 60° C. for optimum growth, a characteristic decreasing the possibility of contamination with mesophilic bacteria. However, these spores could not be recovered from components considered unlikely to be sterilized during manufacture; therefore, a search for a more resistant microorganism was made. Concurrent studies by Koesterer and Bruch (5) and Davis et al. (4) indicated that *Bacillus subtilis* var. *niger* spores were somewhat more resistant to dry heat. Both organisms survived 1 megarad of proton and gamma (Co^{60}) radiation, and were of comparable resistance to volatile liquid carriers. Therefore, *B. subtilis* var. *niger* was used. Acetone was employed as the carrier, since it appeared apparently without effect on the test spores or on most of the sealed components.

Preparation of spore suspensions

The organisms were grown on the surface of nutrient agar containing 0.1 gm./liter of $MnSO_4$ in flat bottles. After 4 or 5 days of incubation at 35° C. for *B. subtilis* and 55° C. for FS1518, growth was harvested, centrifuged, and washed several times with distilled water. The final suspension was made in acetone, with 1 ml. containing approximately 10^7 to 10^8 spores. Spores for the contractor's use were transferred to small amber bottles having screw caps with a rubber membrane.

Selection of components and vendors

Sealed spacecraft components were selected from the Jet Propulsion Laboratories Preferred Parts Lists for extraterrestrial space vehicles. To facilitate selection, components were classified according to their ability to resist thermal exposures as follows:

1. **Heat labile**

a. Heat treatment given during manufacture is unacceptable for sterilization (e.g., hermetically sealed, polystyrene aluminum-foil capacitors—heat-cured 12 hours at 85° C.).

b. These components will sustain permanent damage if exposed to sterilization treatment.

2. **Heat sensitive A**

a. Heat treatment given during manufacture is unacceptable for sterilization (e.g., metal film, epoxy-molded resistors—heat-treated at 150° C. for 10 minutes).

b. These components withstand sterilization treatment without permanent damage.

3. **Heat sensitive B**

a. Heat treatment might be sufficient for sterilization (e.g., wirewound resistors subjected to stress-relieving operation at 125° C. for 48 hours).

b. These components withstand sterilization treatment without permanent damage.

4. **Heat resistant**

a. Heat treatment is sufficient for sterilization (e.g., relays baked at 300° C. for 6 hours, then at 200° C. for 1 hour).

b. These components withstand sterilization treatment without permanent damage.

A list of the components is shown in table I.

TABLE I

Component list

Vendor	Component types	Classification
1 and 2	Wire resistors	Heat sensitive B
3	Carbon-film and metal-film resistors	Heat sensitive A
4	Wirewound epoxy-mold potentiometers	Heat sensitive A
5	Wirewound and carbon-composition potentiometers	Heat sensitive B
6	Germanium diodes	Heat labile
7 and 8	Capacitors	Heat labile and heat sensitive B

Component contamination

After studying the manufacturing procedure, the contractor selected inoculation points at steps in the assembly where maximum contamination could be introduced. By means of a syringe equipped with a 15-gage needle, 1 drop (0.01 ml. — approximately 10^5 to 10^6 spores) of the spore suspension was deposited on the selected site. Special precautions were taken with components containing material that could be damaged by acetone; the spore suspension was deposited as far as possible from the acetone-sensitive area. Contact with the contaminated area was avoided during manufacture. The assembled contaminated components and a report containing pertinent manufacturing information (materials used, curing temperatures, mechanized operations, hand operations, testing performed, and contamination data) were shipped to the School of Aerospace Medicine to be analyzed. Proprietary rights of the manufacturer were, of course, respected.

Bacteriologic analyses

Disassembly of components for recovery of the inoculated spores was accomplished inside a flexible (plastic) film isolator by using tools as shown in figure 1. The isolator and its contents, including the exterior of the components tested, were sterilized with cryoxide gas (ethylene oxide, 11%; fluorinated hydrocarbons, 89%) as described in earlier reports (1, 8). Components were disassembled so that all interior surfaces were exposed for culturing in thioglycollate broth. Cultures of components inoculated with FS1518 were incubated at 55° to 60° C., while those from components inoculated with *B. subtilis* were incubated at 35° C. Incubation was for two weeks, with daily observations for growth (cloudiness of broth). Cultures showing cloudiness were examined by

FIGURE 1

Interior of flexible film isolator.

means of stained smears and were subcultured on tryptone glucose yeast agar.

At the end of the incubation period, all negative cultures were tested for any possible bacteriostatic action of the component material. Four ml. were placed in a sterile tube and inoculated with the spore suspension used to contaminate the component. One ml. was used to prepare dilutions of 10^{-1}, 10^{-2}, and 10^{-3} in thioglycollate broth. These tubes were inoculated similarly. Sterility controls included (a) *B. subtilis* spores dried on filter paper discs and exposed during the sterilization period; (b) tryptone glucose yeast agar exposed in the isolator during the entire performance of the tests; and (c) swabbings of the interior surfaces of the isolator at various intervals during the operation. All tests were cultured in thioglycollate broth at 35° C. for two weeks.

Effects of dry heat on component performance

Tests of the effect of dry heat on performance were done on components not rendered sterile during manufacture. Whenever spores were recovered, a group of similar components was exposed to dry heat at 125° C. for 24 hours. Component performance tests were conducted in the Biomedical Engineering Section before and after the heat treatment. Only undamaged components were examined bacteriologically.

3. RESULTS AND DISCUSSION

The importance and necessity for selecting appropriate microorganisms with high heat resistance is shown with unimpregnated capacitors in table II. Cultures from capacitors contaminated with FS1518 spores during the early part of the program were negative. However, when similar types of capacitors were contaminated with *B. subtilis* var. *niger* spores, positive cultures were obtained. On the other hand, impregnated capacitors yielded negative cultures with either organism.

Results in table II were in agreement with the manufacturing procedures employed. For example, polystyrene types of capacitors were temperature-cured for 12 hours at 85° C. The temperature cure of 12 hours at 125° C. applied to the metallized mylar, and aluminum-foil capacitors were also insufficient to sterilize. These findings confirm the results of Koesterer (6) with dry heat for *B. subtilis* var. *niger* in sand and soil, which required more than 12 hours at 125° C. for sterilization. The manufacturing process appeared sufficient to sterilize the impregnated capacitors. Impregnation of capacitors (e.g., with polybutylene) involves immersing the components in polybutylene solution and subjecting them to a vacuum of 50 μ at a temperature of 125° C. for 24 hours. Studies are in progress to determine the effects of the impregnant solutions, singly and in combination with vacuum, on the thermal resistance of bacterial spores.

TABLE II

Recovery of B. subtilis *var.* niger *and FS1518 spores from capacitors*

Type	FS1518	*B. subtilis*
Unimpregnated		
Polystyrene and aluminum foil	0/7*	6/6
Polystyrene and lead foil	0/6	6/6
Metallized mylar and aluminum	0/3	4/4
Impregnated with polybutylene		
Paper and lead foil	0/3	0/3
Paper, mylar, and aluminum foil	0/3	0/3
Paper and aluminum foil	0/3	0/4
Impregnated with polyester		
Metallized paper aluminum	0/3	0/3
Metallized paper zinc	0/3	0/4
Paper and aluminum foil	0/3	0/4
Impregnated with silicone oil		
Metallized paper zinc	0/3	0/4
Metallized paper aluminum	0/3	0/4
Paper and aluminum foil	0/3	0/4
Impregnated with halowax		
Paper and aluminum foil	0/3	0/4
Paper and lead foil	0/2	0/5

*Number positive/number examined.

As indicated in table III, resistors, potentiometers, and diodes inoculated with FS1518 spores yielded negative cultures. Of those components contaminated with *B. subtilis* var. *niger* spores, only the hermetically sealed resistors yielded positive cultures.

Spores were not recovered from any of the components that were examined after dry heat

TABLE III

Recovery of B. subtilis *var.* niger *and FS1518 spores from resistors, potentiometers, and diodes*

Component type	FS1518	*B. subtilis*
Resistors		
Epoxy-mold carbon film	0/3*	0/8
Epoxy-mold metal film	0/3	0/8
Hermetic-seal carbon film	0/3	13/16
Hermetic-seal metal film	0/3	2/3
Potentiometers		
Resistor carbon	0/5	0/4
Wirewound	0/5	0/5
Diodes-germanium		
GA-5 gold bonded	0/15	Not run
GA-1 point contact	0/10	Not run

*Number positive/number examined.

treatment at 125° C. for 24 hours. The change in performance of components due to this heat treatment is shown in table IV. Changes in resistors, diodes, and potentiometers were within manufacturers' tolerance limits. Capacitance changes varied from short circuiting to less than 1%. As indicated in table IV, the polystyrene and tin-foil capacitors were essentially unaffected; the polystyrene and aluminum-foil types showed marginal changes; the polystyrene and lead-foil types were drastically affected, most of them becoming nonfunctional. Any damaging effects of heat sterilization might be increased if the components were subjected to further heating when installed in circuits of spacecraft instrumentations. Therefore, it is suggested that components rendered

TABLE IV

Performance of components after 24 hours at 125° C.

	FS1518	Percent change		
		Low	High	Average
Resistors				
K10 epoxy mold Carbon film – 1 megohm	4/4*	0.018	0.83	0.25
KC60 hermetic seal Carbon film – 1 megohm	7/7	0.018	0.56	0.22
KMH60 hermetic seal Metal film – 56.5 K ohms	7/7	0.0	0.026	0.014
KM60 epoxy mold Metal film – 56.5 K ohms	7/7	0.008	0.03	0.02
Capacitors				
1P2102 0.001 μf. Polystyrene and tin foil, hermetic seal	4/4	0.75	3.5	2.1
P0012PF 0.001 μf. Polystyrene and tin foil, epoxy and seal	2/2	5.5	5.9	5.6
94-0J184 0.18 μf. Polystyrene and aluminum foil	12/12	2.8	14.8	8.21
94-0J103 0.1 μf. Polystyrene and lead foil	11/11	Shorted		—
YBN36770 0.0059 μf. Polystyrene and lead foil	7/7	8.3	Shorted	—
Potentiometers				
3051L-1-203 20 K ohms trimpot	12/12	0.148	2.26	0.32
Diodes		D.C. reverse leakage change (μa.)		
GA-5 gold bonded germanium	5/5	0.2	8.8	2.3
GA-1 point contact	5/5	0.0	11.5	4.8

*Number changed/number examined.

sterile during manufacture be used wherever possible, thus eliminating the possibility of damaging effects of dry-heat sterilization.

REFERENCES

1. Cordaro, J. T. Studies on the prevention of contamination of extraterrestrial bodies: Bacteriologic examination of hermetically sealed electronic components. SAM Report 62-18, Nov. 1961.

2. Cordaro, J. T., and E. S. Wynne. Sterilization of electronic components of spacecraft. *In* Transactions of the Seventh Symposium of Ballistic Missile and Space Technology, vol. I, pp. 73-82. Aerospace Corporation, Pasadena, Calif., vol. I, pp. 73-82. Aug. 1962.

3. Davies, R. W., and M. G. Comuntzis. The sterilization of space vehicles to prevent extraterrestrial biological contamination. *In* Tenth International Astronautical Congress, Jet Propulsion Lab. External Publ. No. 698, Pasadena, Calif., 1959.

4. Davis, N. W., G. J. Silverman, S. A. Goldblith, and W. H. Keller. Survival of spores at several temperatures in ultrahigh vacuum. Bacteriol. Proc., p. 31 (1962).

5. Koesterer, M. G., and C. W. Bruch. Resistance of dry bacterial spores to sterilization by moist and dry heat. Bacteriol. Proc., p. 30 (1962).

6. Koesterer, M. G. Final Report. Sterilization of space probe components. NASA Contract NASr-31 (1962).

7. Phillips, C. R., and R. K. Hoffman. Sterilization of interplanetary vehicles. Science 132:991-995 (1960).

8. Wynne, E. S. Sterilization of space vehicles: The problem of mutual contamination. *In* Lectures in Aerospace Medicine, Jan. 1961, USAF Aerospace Medical Center, Brooks AFB, Tex.

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